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—NEW FINDINGS—

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LABILE METHYL GROUPS IN NUTRITION

–NEW FINDINGS–

The discovery six years ago of transmethylation as a newly recognized process in metabolism, marked an important milestone in nutrition research. The concept of biological transmethylation is based on the existence of a hitherto unsuspected dietary essential—the labile methyl group. The methyl group ($-CH_3$), simplest radical grouping known to organic chemists, constitutes an integral part of many common organic compounds. In most of these the methyl group is firmly bound within the molecule; in certain others, however, it possesses the property of being "labile" or unstable. Methyl groups which possess this property of lability are capable of being shifted from one compound to another by simple molecular interchange. Such interchange or transfer of labile methyl groups from one compound to another is described as *transmethylation,* a process which has been shown to have considerable import in biological mechanisms.

The labile methyl group is of the same biological significance as the vitamins. Like the vitamins, this grouping constitutes a nutritionally indispensable unit which cannot ordinarily be synthesized in the body and which must, therefore, be provided in the diet. The major significance of the labile methyl group lies in its relation to other nutritionally important factors such as the sulfur-containing amino acids and the B-complex vitamin, choline. All of these materials have been shown to be intimately related to liver and kidney function as well as to the general processes of life and growth.

Recent advances in the study of transmethylation processes have established several interesting new facts. Among the most significant has been the demonstration that transmethylation of compounds occurs as a normal metabolic process in human beings as well as in experimental animals. This discovery has direct practical implications in nutrition and metabolism studies and gives promise of having significance in certain phases of liver and kidney dysfunctions. A further development is the very recent discovery that labile methyl groups again like the vitamins, may be synthesized, under specialized conditions, by bacterial action in the intestinal tract.

The existence of methyl transfer has been the means of explaining many hitherto unsolved problems in the intermediary metabolism of sulfur, nitrogen, fat and carbohydrate. Some of the newer findings in these fields are described in this *Review* in an attempt to clarify the almost universal role of the transmethylation phenomenon in metabolic processes.

Discovery of Transmethylation:

The discovery that certain methylated compounds contain freely transferable $-CH_3$ groups which play an important role in biological processes came about through early studies on the nutritional role of the various amino acids. The story of this discovery has been described in detail in a previous issue of this *Review,** and will be reviewed but briefly here.

During an investigation of the nutritional indispensability of the three related sulfur-containing amino acids—cystine, homocystine, and methionine—an inexplicable discrepancy in findings was brought to light as a result of similar experiments conducted by two independent groups of investigators. At the University of Illinois, a group of researchers headed by Rose (1), pioneer investigator of the nutritional importance of the amino acids, found that the essential amino acid, methionine, could apparently be replaced in animal nutrition by homocystine, one of the other two related sulfur amino acids previously mentioned.

During the same year, 1939, du Vigneaud and associates, working independently on the same problem at Washington University (2), found that neither homocystine nor cystine could successfully be substituted for methionine in the diet of laboratory animals. While Rose's animals thrived successfully with homocystine replacing methionine, du Vigneaud's grew thin and listless and eventually developed pathological fatty livers on the homocystine diet. In checking details of their respective experiments the two groups found one important difference in the experimental conditions: Rose's group had used as part of the basal diet, vitamin concentrates from milk or rice polishings, both natural sources of the B complex, while du Vigneaud's group had provided each member of the B complex separately in the form of synthetic thiamine, riboflavin, nicotinic acid, etc. Obviously then, the natural concentrates from milk or rice polishings were providing along with the B complex an additional, unidentified substance which enabled the non-essential amino acid, homocystine, to replace the essential methionine.

The appearance of pathological yellow livers in the animals on the synthetic vitamin diet provided a clue to the identity of this mysterious factor. For a long time it had been known that fatty infiltration of the liver could be prevented or controlled in experimental animals by choline, a simple component of lecithin, containing three methyl groups in its molecular structure. Now at Cornell University, du Vigneaud and a new group of associates (3) followed the problem further. The addition of choline to the methionine-free diet containing homocystine not only cleared up the fatty liver condi-

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**cf.—Borden's Review of Nutrition Research,* October, 1942—"Labile Methyl Groups of Protein and Choline."

tions, but restored growth to the normal level which might be expected had methionine been returned to the diet.

At this point, du Vigneaud's group made some very interesting deductions along the following lines: (1) Animals receiving homocystine in place of methionine fail to grow unless choline is added to the diet. This suggested and was, indeed, later confirmed by analysis, that choline was probably the mysterious factor in the natural B complex sources of Rose's diet which protected his animals from showing signs of methionine deficiency and permitted them to grow with homocystine. (2) The only difference between methionine and homocystine is the presence of a methyl group in the former compound. (3) Choline is a compound containing methyl groups. (4) It seemed possible that choline provided homocystine with the necessary methyl group to convert it into methionine. Here indeed was a thought provoking conclusion! A simple experiment soon demonstrated the validity of this assumption. The ethyl analogue of choline, triethylcholine (containing three ethyl groups in place of the three methyl groups), although capable of choline-like activity with respect to curing fatty livers, had no effect in restoring normal growth of methionine-deficient animals.

Thus, what set out originally to be the clarification of a small, but puzzling discrepancy in scientific procedure, unexpectedly led du Vigneaud's

Synthesis of Methionine from Homocystine and Choline by Methyl Transfer (Transmethylation)

Homocysteine*	Choline	Methionine
S-H	CH_3	S-CH_3
|	|	|
CH_2	HO-N-CH_2-CH_2-OH	CH_2
|	||	|
CH_2	$(CH_3)_2$	$\rightleftharpoons$ CH_2
|		|
$HCNH_2$		$HCNH_2$
|	↓ [CH_3] ↑	|
COOH		COOH

FIG. 1. Homocysteine + Labile Methyl → Methionine

*For purposes of simplicity, the formula of homocysteine has been used. Two molecules of homocysteine combine to form one molecule of homocystine.

group (3) to the enunciation of a new theory in biochemistry. This theory proposed that choline, and probably other methylated compounds as well, may serve as a donor of methyl groups to permit the synthesis in the body of essential methionine from homocystine provided in the diet (Fig. 1). A corollary to this theory postulated that methyl groups in a utilizable, labile form cannot be synthesized by the body and must, therefore, be provided in the diet in order to make possible essential methylation procedures such as the conversion of homocystine to methionine.

With the availability of isotopic hydrogen, deuterium, it became a relatively simple matter to trace the metabolic pathways of "tagged" methionine or choline and to demonstrate conclusively the occurrence of a reversible methyl transfer between these two compounds* (4, 5).

Significance of the Transmethylation Process:

With the conclusive demonstration that methyl transfer is a fundamental biologic process requiring for its effective functioning a dietary supply of labile methyl groups, new perspective was given to the interpretation of many basic biochemical phenomena. The question now arises: What significance does the transmethylation process have in the animal economy or, to put it another way, to what uses may labile methyl groups provided in the diet be put by the living organism? At the time of the enunciation of the transmethylation concept, it was already clear that biological synthesis of methionine from choline or vice versa was one important answer to this question. In the light of additional experimental studies, it has become apparent that methyl transfer is a significant step in many other widely varying phases of intermediary metabolism including nitrogen and sulfur metabolism, fat metabolism and carbohydrate metabolism.

Methionine—Choline—Homocystine: The original discovery of a metabolic interrelationship between choline and the amino acids methionine and homocystine through the interchange of methyl groups itself developed many ramifications into other fields. Perhaps the most significant of these is the linking of these compounds with the phenomenon of lipotropic and anti-hemmorhagic activity. A lipotropic compound is one which is capable of preventing or correcting excessive accumulation of lipid or fat material in the liver. As has already been pointed out, choline had long been known to possess such activity, having been found by Best *et al* to be capable of preventing a dietary induced condition of pathological fatty liver (6, 7).

Further investigation by other workers on dietary factors which affected

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**cf.—Borden's Review of Nutrition Research,* October, 1942, December, 1945.

fatty infiltration of the liver revealed that the milk protein, casein, (8, 9) and specifically the amino acid, methionine (10), were also valuable lipotropic agents. Strangely enough, the closely related amino acid, homocystine, was found to have no lipotropic activity (11, 12). A puzzling situation: Two almost identical amino acids exhibited profound differences in lipotropic behavior, whereas two apparently totally unrelated compounds as methionine and choline were extremely similar in this particular biological action.

Interpreted in the light of the new transmethylation concept, the explanation became surprisingly simple. Methionine, by supplying a source of labile methyl groups, permitted the body to synthesize choline; therefore, providing methionine became equivalent, ultimately, to providing choline. Homocystine, on the other hand, having no methyl groups, could not give rise to choline and hence was of no lipotropic value. The lipotropic activity of casein, of course, is derived from its high methionine content. All of these compounds have also been found to prevent the dietary deficiency syndrome of hemorrhagic kidneys in experimental animals (13, 14, 15).

The mechanism involved in the prevention of fatty livers by choline has already been described, as has been its relation to the hemorrhagic kidney disorder.* The relationship between these activities and the presence of a supply of labile methyl is an intriguing one. As du Vigneaud said (16):

> "All of the methyl donors established by the growth criterion are effective in preventing these deficiency syndromes *(fatty liver and hemorrhagic kidneys)* but there are many lipotropic and antihemorrhagic agents which are not methyl donors by the growth criterion. An interesting example of this type is arsenocholine studied by Welch (17). Furthermore, compounds not even containing methyl groups can prevent fatty livers and hemorrhagic kidneys. For example, triethylcholine . . . We come then to the realization that the possession of a labile methyl group *per se* cannot be called upon to explain the effectiveness of choline in these functions. An agent may be effective without regard to lability of the methyl or the presence of a methyl—but to reiterate, any compound which is a methyl donor will be effective by making possible the synthesis of choline. Although methyl donors are not unique in preventing these deficiency symptoms, the deficiency symptoms are indicative of a lack of methyl donors ordinarily present in the diet. . . . The reason for the lipotropic (and antihemorrhagic) effect of choline itself is by no means clear."

Methionine-Cystine: The elucidation of problems in sulfur metabolism, more specifically in the conversion in the body of methionine to cystine, has been greatly facilitated by the knowledge that methyl transfer exists as a normal biological function. It has been an accepted fact for some time that methionine can be converted to the essential amino acid cystine in the body. How this occurs has long puzzled biochemists. Many intricate and ingenious

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**cf.—Borden's Review of Nutrition Research,* October 1942—"Labile Methyl Groups of Protein and Choline."

experiments were devised to test the various theories on this subject and the findings of many independent investigators (18, 19, 20, 21, 22, 23) were correlated to provide highly convincing evidence of what the actual metabolic pathways are in this conversion. Briefly, the conclusions seem to indicate that methionine by giving up its labile methyl group becomes converted to homocysteine (Fig. 1). By combining homocysteine with another amino acid, serine, it becomes possible for the original sulfur of methionine to become an integral part of a new compound which then undergoes cleavage to eventually yield the new sulfur amino acid, cystine.

Creatine Formation: The demethylation of methionine as a preliminary step in the formation of cystine makes available to the body a liberated methyl group. The fate of this methyl group and the possibility that it might be utilized for specific methylation processes in the body opened an additional avenue of research in the transmethylation problem. The possibility was suggested by Lewis (24) and Brand *et al* (25) that the liberated methyl group of methionine might be used in the methylation of nitrogen in creatine formation. Creatine when combined with phosphorus, plays a very important role in the intermediary metabolism of carbohydrate in muscle tissues. For this reason, a search for the precursors involved in the biological origin of creatine has long occupied the attention of biochemists. A search which began almost twenty years ago had finally culminated in the demonstration that the immediate precursor of creatine is guanido acetic acid, a compound which is identical with creatine except for the absence of a methyl group. The origin of guanido acetic acid, in turn, has been traced by ingenious isotope (26) and tissue slice (27) experiments to a combination of the amino acid glycine with a portion of the amino acid arginine. Thus, the picture of creatine synthesis was complete except for the question as to how guanido acetic acid became methylated to form creatine.

The suggestion that the methyl group of methionine might be utilized for this purpose (in addition to its already demonstrated function in choline synthesis) was examined experimentally. In the tests where isotopically tagged methionine had been fed to determine its role in choline synthesis, du Vigneaud *et al* (4, 5) took the opportunity at the same time of analyzing samples of isolated tissue creatine and urinary creatinine to see whether any of the deuterium from the "tagged" methyl group of methionine had found its way into the creatine molecule. Positive results indicated fully that the final link in the chain of the creatine synthesis story had been found.*

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*Feeding isotopically marked choline also led to the formation of isotopic creatine, but to a much lesser extent than that which followed the feeding of methionine. This was eventually interpreted as an indication of the preliminary conversion of choline to methionine before its methyl groups find their way into the creatine molecule.

The metabolic relationships between the various methyl compounds thus far discussed have been summarized simply in the following schematic arrangement given by du Vigneaud (16):

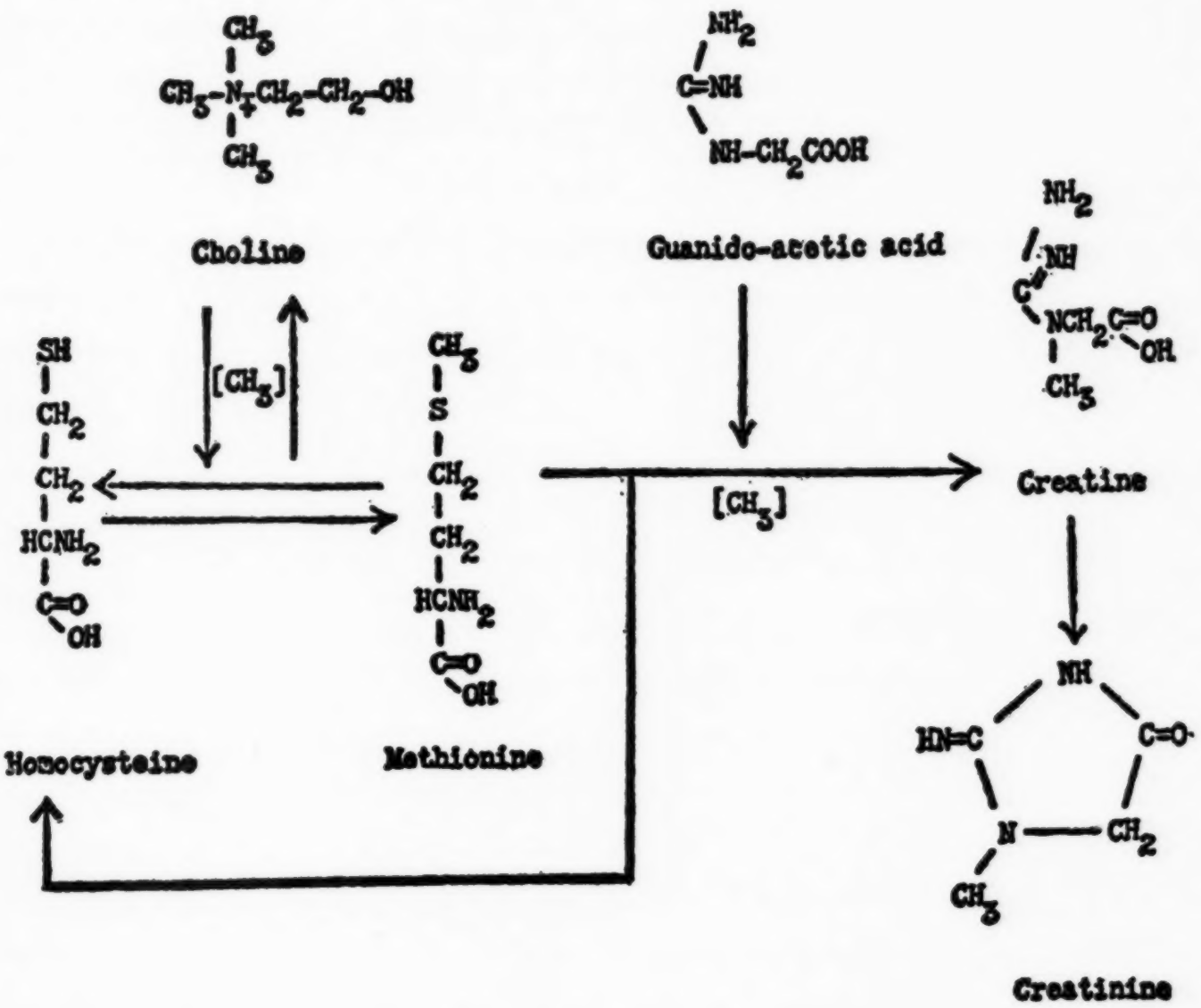

FIG. 2. Scheme of the metabolic interrelationships of methionine, choline and creatine.

Other Metabolic Pathways: There are more than thirty compounds known to exist in or to be excreted by the animal body which possess nitrogen or sulfur-bound methyl groups or which are in some way structurally related to choline. Many of these such as creatine, adrenalin, anserine, trigonelline, methyl pyridine, etc., are known to have extremely important roles in metabolism; the function of certain others remains somewhat obscure. Nevertheless, the possibility exists that many, if perhaps not all, of these materials are significantly interrelated through their methyl groups and, for that reason, their occurrence and subsequent effect on body functioning may be intimately involved with the supply of dietary methyl. The possibility of dietary methyl influencing the production of, for example, adrenalin is a fascinating one. Similarly provocative is the theory of a "labile methyl deficiency" in pellagra, or of the significance of the dietary methyl supply in vital detoxification mechanisms involving methylation procedures (5).

Biosynthesis of Labile Methyl:

The possibility exists that, as with the vitamins, a certain small proportion of the labile methyl requirement may be synthesized in the body by bacterial action in the intestinal tract. Early in their studies, du Vigneaud, Chandler, Moyer and Keppel (3) noted that an occasional animal was capable of showing some growth on a diet containing homocystine, but neither methionine or choline. They suggested that the growth was due to refection similar to that which occurs in intestinal synthesis of the B vitamins.* Last year it was actually demonstrated by the use of isotopes that a small, but not insignificant, amount of labile methyl groups had been synthesized in rats on a diet containing an adequate supply of methyl groups in the form of methionine (31). The synthesis was thought to be due mainly to the activity of intestinal bacteria. It has been concluded, however, that in general such synthesis is insufficient to provide for the essential methyl requirement or to sustain growth in animals on a homocystine diet devoid of methionine and choline.

An unusual report by Bennett, Medes and Toennies (32), however, indicates that essential methionine and cystine seem to be synthesized from homocystine even in the dietary absence of any known source of nitrogen or sulfur-bound methyl groups in quantities sufficient to sustain growth. In their studies, young animals were maintained on a synthetic diet of amino acids (excluding methionine and cystine), vitamins and other essentials (excluding choline) presumably quite free of labile methyl groups. On this diet the animals rapidly lost weight. The animals were then divided into separate experimental groups in order to determine the growth response to varying amounts of supplementary homocystine, cystine, methionine and choline. In the homocystine-supplemented group practically all of the animals responded with good weight gains when provided with liberal amounts of this amino acid.

After several months about 20 per cent of the animals ceased to gain weight or began to lose slowly; about one-third developed a curious illness from which, despite the severity of symptoms, they eventually made spontaneous recoveries. Analysis of the tissues of sacrificed animals which had been fed the homocystine-supplemented diet compared favorably with that of the tissues of normal control animals with respect to methionine and cystine contents (33). Since the animals had grown, it was concluded that the methionine and cystine found in the tissues must have been synthesized from homocystine in the absence of choline. Several possible explanations

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**cf.—Borden's Review of Nutrition Research,* May 1946—"The Biosynthesis of Vitamins."

were offered including the existence in the pre-experimental diet of unknown combinations of labile methyl groups which had then been stored in reserve depots; intestinal synthesis of labile methyl; or the fact that certain vitamins may have enabled the animal to compensate for the lack of dietary methyl by stimulating biosynthesis.

Transmethylation in Man:

Investigations into the occurrence of transmethylation reactions in animal species other than the laboratory rat have shown that this phenomenon occurs in the chick (28, 29), the rabbit (16) and, most significantly, in man. The studies leading to the establishment of the transmethylation process in man were conducted only two years after the original discovery of biological methyl transfer and made use of the new tool of isotopic tracers as a means of checking results. In these tests (30), deuterium-labeled methionine was fed to a normal adult male over a period of several days. Creatinine isolated from the urine and choline extracted from blood samples contained sufficient concentrations of deuterium in their respective methyl groups to indicate that the source was the dietary methionine (Table 1).

Table 1. Feeding of Deuteriomethionine to Man

Time	Compound isolated	Atom per cent D in methyl group	Percentage methyl groups derived from dietary methionine
HOURS			
1—24	Urinary Creatinine	0.12±.05	0.16±.06
25—48	Urinary Creatinine	0.30±.05	0.41±.06
49—72	Urinary Creatinine	0.40±.05	0.54±.06
72*	Blood Choline	1.38±.09	1.9 ±.1

*Blood sample taken at 72nd hour.

Practical Implications of Methyl Transfer in Man: A study of the various biological tissue components and excretion products containing potentially labile methyl groups, as mentioned on page 7, permits some indication of the possible scope of the transmethylation process in the human economy. Some possible relationships have already been mentioned in this connection. A further possibility which has been suggested in the light of the role of methionine in muscle creatine synthesis is the consideration of methionine as a factor in muscular dystrophy.

The suggestion that a deficiency of labile methyl may be an important factor in the etiology of pellagra (34) presents a number of thought-provoking possibilities. For example, it was recently pointed out by Woolley (35) that the long-recognized "toxic" effect of corn diets in precipitating the occurrence of pellagra even when nicotinic acid is provided, is due to the presence in corn of a structural analogue of nicotinic acid. This analogue, apparently by exerting a competitive effect on the enzyme systems involved in the utilization of nicotinic acid, acts, in effect, as an "anti-nicotinic acid," thereby precipitating symptoms of nicotinic acid deficiency, i.e. pellagra. Woolley's experiments have revealed this pellagragenic substance to be a pyridine compound. Among the labile-containing biological compounds listed by du Vigneaud is the compound methyl pyridine. The thought presents itself that an excess of pyridine compounds in the body may compete with nicotinic acid to produce pellagra *unless they are "detoxified" by being methylated* and thereby removed from such competition. This might explain the relation of methyl deficiency to the development of pellagra.

Aside from these fascinating speculations concerning the intricate relationships among the various methylated compounds in the body, there remain the practical implications in the use of choline, methionine and cystine as therapeutic agents in the treatment of human liver disorders. These include fatty, enlarged livers, liver cirrhosis and possibly infectious hepatitis. The role of choline and methionine as lipotropic agents has already been discussed. Experiments have established beyond doubt that the lipotropic factors are intimately concerned with dietary cirrhosis. György and Goldblatt (36) have stressed the fact that the factors predisposing to fatty livers—high fat, low protein diets, the absence of choline and other lipotropic substances—are the same as those which in more prolonged experiments lead to necrosis and cirrhosis. Further, the preventive and curative measures are the same for both fatty livers and cirrhosis—casein, methionine and choline.

Although the transition from animal experimentation to clinical medicine is often a slow and indirect process, encouraging reports have already been forthcoming with respect to the control of corresponding human liver diseases by dietary means. The similarity between the animal and human diseases has not always been precise. Nevertheless, starting with the work of Patek in 1937 (37) and in 1941, together with Post (38), reports of the successful dietary management of human liver disease have been increasing. Choline, casein and methionine—major dietary sources of labile methyl—are today recognized and accepted as important factors in the management of liver cirrhosis. The theory behind this acceptance remains fraught with near-solutions—a challenge to the continuing research in this field.

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